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(19)


## (54) A CELL FOR ELECTRO-CHEMICAL ANALYSIS

(71) We, RADIOMETER A/S, a company organized under the laws of Denmark, of No. 72, Emdrupvej, DK 2400 Copenhagen NV.—, Denmark, do hereby declare the invention, for which we pray that a patent may be granted to us, and the method by which it is to be performed, to be particularly described in and by the following statement:—

The present invention relates to a cell for electro-chemical analysis and, more particularly, to such cells which comprise an enzyme for converting the substance to be measured into a product which, in a correlated way, can influence the electrical signal from the cell.

Such cells comprise, in a receptacle, an indicator or sensing electrode electrically contacting one side of a membrane and a reference electrode electrically contacting the same side of said membrane. In this context the expression "contacting" covers the case in which contact is obtained through a layer of electrolyte.

In the case of an enzyme membrane structure a second, hydrophilic membrane is arranged at a distance from the first-mentioned membrane and in the space between the two membranes a layer of concentrated enzyme is present. The free face of the second membrane provides the test surface to which the substance to be tested is applied. An enzyme membrane of this is described in the Annals of New York Academy of Science 102, pages 29—49, (1962).

The first-mentioned membrane facing the sensing electrode is made of a material which can be penetrated by the substance to which the sensing electrode is sensitive. Thus this membrane is permeable to the reactants of the enzymatic reaction but impermeable to enzymes. It may be made of cuprophane, but in the event that one of the reaction products is a gas at normal pressure and temperature and it is desired to measure via this gas, the membrane may consist of hydrophobic plastics impermeable to ions but slightly permeable to such gases as oxygen, carbon dioxide or ammonia. Known plastics having such properties are silicone rubber, polytetrafluorethylene, polypropylene etc.

Biological fluids and other samples to be tested may contain substances that may interfere with the sensing electrode. In a polarographic cell substances such as uric acid and ascorbic acid may be oxidated on the anode and result in a current signal which is not due to the substance to be measured and which is therefore not correlated to the quantity of this substance. In order to avoid this difficulty it has been suggested in British patent specification 1,167,317 to use the current from a second cell which is identical with the enzyme electrode cell but for the lack of enzyme in the membrane to compensate the error in the current from the enzyme electrode cell. However, producing error signals of identical magnitude in different cells is almost impossible and, therefore, this arrangement presents further practical problems.

It is an object of the present invention to devise a cell for electro-chemical analysis in which error signals of the kind described are avoided in a simple but effective way without affecting the practical applicability of the cell.

According to the present invention the problem encountered is solved by means of a cell for electro-chemical analysis which comprises a receptacle, a sensing electrode in said receptacle, a reference electrode in a space in said receptacle which is separated from said sensing electrode and adapted to hold an electrolyte solution, and a composite membrane electrically contacting said sensing electrode, a path for an electric current extending between the reference electrode and the sensing electrode, said composite membrane comprising at least two strata, one of which has a lower density than the other and is chosen to the sensing electrode than the other and the other of which has a thickness which is only a small fraction of the total thickness of the membrane. This cell can be used for polarographic as well as potentiometric measurements.

The composite membrane is an inhomogeneous membrane formed as a unit and hav-

ing different properties in different strata parallel to the surface of the membrane. This membrane blocks the migration to the sensing electrode of interfering substances, such as uric acid, ascorbic acid, ions and non-gaseous molecules and similar substances and allows the passing of relevant substances, for example, an enzymatic conversion product such as hydrogen peroxide or a gas, for example oxygen, carbon dioxide or ammonia.

A homogeneous membrane exhibiting these properties can be made of silicone rubber. However, in order to obtain a reasonable short measuring time, experience has shown that the thickness of the membrane should not exceed 0.2—5 μm depending on the kind of measurement to be carried out. It would then be possible to achieve, in a practically acceptable short response time, an equilibrium of diffusion of, for example, hydrogen peroxide. Unfortunately, a membrane of this thickness is not practically applicable owing to the lack of mechanical strength.

The above-mentioned problem has been solved in accordance with the preferred forms of the invention by applying a composite membrane with at least two different strata, one of which has a thickness less than 5 μm, preferably between 0.01 and 2 μm, when the substance migrating through the dense strata and depolarizing the anode is hydrogen peroxide and 0.1 to 5 μm when said substance is a gas, while the other stratum is sufficiently thick to render the necessary mechanical strength. The thin, dense stratum may be a hydrophobic material such as silicone rubber or a hydrophilic material such as cellulose acetate and the thick and less dense stratum may be a hydrophilic material, such as cuprophane, cellulose acetate or polymerised protein.

It has been found that a cellulose acetate membrane for reverse osmosis marketed by the Danish company "De danske Sukkerfabrikker" under the code number "999" is likewise applicable as a membrane in a cell according to the present invention.

This membrane "999" consists entirely of cellulose acetate, having dense and less dense strata. The membrane is manufactured by dissolving cellulose acetate in a mixture of formamide and acetone. This solution is spread onto a plate and the solvent is partly evaporated in 5—10 seconds, after which time the plate is cooled in icecold water for 30 minutes. On the membrane produced a skin-layer forms on that side which during the evaporation has been exposed to the air. This skin-layer is made more or less dense by hot water treatment at 80—90°C., high temperature and long treatment time giving a more dense layer.

In order that the invention may be more readily understood reference will now be made to the accompanying schematic drawings, in which

Fig. 1, in vertical section, shows an embodiment of a cell according to the invention,

Fig. 2 shows a view from the bottom of the cell shown in Fig. 1 with the membranes removed,

Fig. 3 shows an enlarged view of that part of the cell which is shown within the circle in Fig. 1,

Fig. 4, in vertical section, shows a part of another embodiment of the cell according to the invention comprising a glass electrode, and

Figs. 5 to 8, in vertical section, show four different embodiments of an enlarged part of a composite membrane for a cell according to the invention.

Fig. 1 shows one way of exploiting the present invention in connection with an enzyme cell. The cell comprises a receptacle having a cylindrical jacket 3 which is closed by a cap 3a, preferably of electrically insulating material at one end and by a membrane structure 6 at the other end.

Inside the receptacle is a central cylindrical supporting column 5 for an indicator or sensing electrode 4 acting e.g. as anode in the cell. The receptacle 3, 3a and the supporting column 5 may be of glass or plastics.

The active part of the anode 4 may be of, for example, platinum, gold, silver, graphite. In the case of a platinum anode, the surface in contact with the electrolyte solution 2 may be covered by platinum black. The electrolyte may include sodium chloride or potassium chloride, buffers including carbonate, phosphate, bicarbonate, acetates, alkali or alkali earth metal salts, or other inorganic or organic buffers or mixtures thereof. The solvent for such electrolytes may be water, glycols, glycerine, and mixtures thereof. The support column 5 may be hollow as indicated by an empty space 17. Between the jacket 3 and the support column 5 there is a cylindrical space which extends down to the membrane structure and is partly filled with a suitable electrolyte 2 in which is immersed a reference electrode 1 acting as a cathode (although in some cells anode and cathode may be transposed), which may be silver chloride coated silver wire.

The electrodes 1 and 4, are, through leads 12 and 11, respectively, connected to an apparatus 20 comprising a d.c. voltage source, not shown, and a d.c. current measuring instrument A.

The receptacle is provided with a vent 10 in the jacket 3 closed by a rubber band 9 permitting gas to escape if the pressure inside the receptacle rises to a sufficiently high level.

The membrane structure 6 covering the open end of the receptacle 3, 3a is kept in place by means of an O-ring 7 fitting in a cir-

cular groove 8 in the outer surface of the jacket 3 close to the bottom.

Fig. 2, which shows the receptacle 3, 3a seen from the bottom after removal of the O-ring 7 and the membrane structure 6, illustrates that the current path extends radially out from the central anode 4 to the circular electrolyte 2 surrounding the anode 4. However, the geometrical form of the cell is not important and many other embodiments are feasible in carrying out the present invention, which mainly resides in the special membrane structure. The membrane can be used in connection with any electrochemical cell of that type claimed in claim 1 and of any design including an electrode sensitive to light molecules or ions derived from the substance to be analysed directly or through enzymatic conversion.

The part of the cell shown inside the circle in Fig. 1 is shown enlarged in Fig. 3. In this embodiment the membrane structure 6 comprises a composite, non-homogeneous membrane having two strata 13 and 16, an enzyme layer 14 and a membrane 15, the outer free surface of which represents the test surface which is to be brought into contact with the solution 18 to be analysed, which can be present in a vessel, not shown.

The composite, non-homogeneous membrane has a thin, dense stratum 16 and a thick, less dense stratum 13 which strata together form a unity. In order not to extend the measuring time to a value which is unacceptable in practice the dense stratum must be very thin, viz. 0.01 to 5 $\mu$m. The mechanical strength of a single stratum membrane that thin would be insufficient for practical use.

It has been found, however, that a membrane having a stratum of cuprophane and a thin, dense stratum of silicone rubber solves the problems relating to selective permeability and mechanical strength.

A method for producing a composite membrane may consist in applying a thin layer of a mixture of dichlordimethylsilan and trichlormethylsilan on one or both sides of a sheet of cuprophane, the thickness of which is 10 to 100 $\mu$m. By adding water, $H_2O$, a hydrolysis occurs and dihydroxyldimethylsilan, trihydroxymethylsilan and hydrochloric acid is formed. By heat treatment dihydroxyldimethylsilan and trihydroxymethylsilan is polymerised under the elimination of water into a silicon rubber bonded to the cuprophane. The stratum added should have a thickness of 0.01 to 2 $\mu$m, when hydrogen peroxide is the active depolarizing agent.

The advantage of bonding the dense stratum to the stratum or substrate acting as a carrier is, that it does not wear off, a property which, for many reasons, is important in practical use.

Instead of cuprophane other hydrophilic carriers such as cellulose acetate, polymerised protein and other substances (which meet the requirements of claim 1) may be used as part of the non-homogeneous membrane.

The non-homogeneous composite membrane need not consist of two strata of different materials. What is needed is a membrane having two strata of different properties, (i.e. a denser stratum and a less dense stratum with the denser stratum having a thickness which is only a small fraction of the total thicknes of the membrane), one of which has a high permeability to light molecules such as hydrogen peroxide and a very low permeability to ions and neutral molecules with a high molecular weight. In a cellulose acetate membrane different properties in different strata can be obtained by heat treatment. A membrane material of this kind with exceptionally good properties is commercially available from the Danish company "De danske Sukkerfabrikker" under the code number "999" and has already been described. The surface of the cellulose acetate membrane has been heat treated whereby a dense stratum with a thickness of 0.1 to 1 $\mu$m is formed in a sheet which is approximately 120 $\mu$m thick.

A dense stratum can be formed on both sides of the sheet or as an intermediate layer by a short-time high frequency heat treatment of a sheet between cooled condenser plates.

In Figs. 5—8, are schematically illustrated some of the possible configurations of strata in the composite membrane. It is obvious that the thicknesses of the strata cannot be shown correctly in a case in which the ratio between the dense and the less dense stratum is 1:100.

In Fig. 5, a dense stratum 21 and 22 is provided on both surfaces of the sheet leaving a less dense stratum 23 in the middle. In the application of this membrane in which the dense stratum may have hydrophobic properties, a thin layer of electrolyte should be allowed to form between the sensing electrode and the membrane.

In Figs. 6, 7 and 8, a hydrophilic stratum 24, 28 and 31, respectively, covers the surface of the membrane facing the sensing electrode which may, therefore, touch the membrane.

In Fig. 6 a second hydrophilic stratum 26 is arranged between two dense strata 25 and 27.

The membrane in Fig. 7 has a stratum 28 of less dense hydrophilic material and a stratum 29 of dense hydrophobic material. This membrane may be used in a cell for detecting gas such as ammonia, hydrogen sulphides, etc., in a fluid or gaseous sample. In Fig. 7 is further illustrated the possibility that an electrode 30 is applied directly on to the stratum 28 of the membrane, for example, by coating. An enzyme may be included in the stratum 28 when it is to be used without the electrode 30.

By bonding an enzyme chemically or mechanically either on to the outer surface of the membrane and in contact with a test solution or on to the inner surface of the membrane, the cell can be used for testing substances in which the conversion product has a molecular weight below 500 and can be detected by the sensing electrode. Suitable membrane materials are polymerised protein, cuprophane, cellulose acetate and similar hydrophilic materials.

Depending on the analysis to be carried out, the stratum 33 in Fig. 8 can likewise be with or without an enzyme.

In an embodiment of the cell (not illustrated) the denser stratum contacts an aqueous layer of enzyme contained between said stratum and a second, hydrophilic membrane kept at a distance from said stratum by at least one spacer. The spacer prevents the aqueous enzyme solution being pressed away from the surface of the denser membrane when the hydrophilic membrane is subject to a pressure from the test solution.

In another embodiment (not illustrated) said denser stratum contacts a spacer containing enzyme which keeps a second membrane at a distance from said stratum, said second membrane being permeable to molecules and ions of lower molecular weight only. In this case, the enzyme layer per se acts as a spacer so that the pressure of the test solution will not remove the enzymes from the space between the denser membrane and said second membrane.

The enzyme electrode cell can, for example, measure the glucose content in a sample by means of the enzyme glucose oxidase which selectively catalyses the following reaction.

$$\text{Glucose} + O_2 \xrightarrow{\text{Glucose oxidase}} \text{Gluconic acid} + H_2O_2$$

The sensing electrode in the cell is sensitive to hydrogen peroxide which gives rise to an electrical current signal which is proportional to the amount of hydrogen peroxide, which in turn is proportional to the concentration of glucose in the test solution. Thus, the cell can be used for measuring the glucose content in biological fluids such as blood and serum. These biological fluids contain many substances that are capable of being oxidated directly on the sensing electrode, but such substances, for example, uric acid, ascorbic acid and certain drugs, are prevented from reaching the sensing electrode by the dense stratum in the composite membrane.

The use of oxidases and anodic determination of peroxide is only mentioned as an example. The invention may be used also where an enzyme, or even an inorganic catalyst, converts a diffusible electro-chemically inert substance into an electro-chemically active substance. Mixtures of enzymes or different enzymes in different layers or strata in the membrane structure may be used where more than one reaction is required to convert the substance to be measured into an electro-chemically active product.

The cell shown in Fig. 4 may be used for potentiometric measurements and comprises a receptacle 40 of electrically insulating material with two compartments 41 and 42 of which 41 is annular and contains an electrolyte with a reference electrode 43, and 42 is cylindrical and contains a reference electrolyte with an inner-reference electrode 44. The electrolyte may be any conventional electrolyte used in connection with the reference electrode 44 of e.g. Ag/AgCl such as NaCl. In the case of a pH sensitive membrane ($H^+$ sensitive) being applied a pH buffer for example a citrate having a pH of 5.5 is added. The compartment 42 is closed at the bottom by an ion sensitive membrane e.g. a glass membrane 45 which is sensitive to $H^+$ ions. Depending on the measurement to be carried out membranes may be used which are sensitive to other ions such as $Ag^+$, $S^{2-}$, $F^-$, $Cu^{2+}$ and $Cl^-$.

The bottom of the receptacle 40 is covered by a non-homogeneous composite membrane which may be kept in place in any suitable way for example in the same manner as shown in Fig. 1. This membrane 46 comprises two strata, one of which is a less dense stratum facing the glass electrode 45 and the compartment 41 with the electrolyte and the other of which is a denser stratum, which is impermeable to ions, facing the medium to be tested, the denser stratum having a thickness which is only a small fraction of the total thickness of the membrane.

The electrodes 43 and 44 are through leads connected to an electrical measuring instrument for measuring the voltage. The input resistance may be $10^{10}$—$10^{14}$ ohm.

The cell provided with a pH-sensitive glass membrane may be used for measuring $NH_3$ concentration in a medium to be tested. As an electrolyte in the annular compartment is used

$$NH_4Cl \leftrightharpoons NH_4^+ + Cl^-$$

which in equilibrium with $NH_3$ results in

$$NH_4^+ \leftrightharpoons NH_3 + H^+ \Rightarrow \frac{[H^+]\ [NH_3]}{[NH_4^+]} = \text{constant.}$$

Owing to the fact that the ratio between the concentrations

$$[H^+] \quad [NH_3] \quad \text{and} \quad [NH_4^+],$$

and consequently the potential of the electrode chain in the cell, is substantially constant a voltage signal will occur corresponding to the concentration of $NH_3$ in the medium to be tested because the $H^+$ ion concentration diminishes as the $NH_3$ concentration increases while the $NH_4$ concentration remains substantially constant. This medium may have a pH different from that of the electrolyte in the annular compartment 41 because no ions, including $H^+$ ions, can penetrate the dense stratum of the membrane, 46, e.g. silicon rubber, while the gas $HN_3$ passes through.

As another example in which a gas $NH_3$ is used for measuring may be mentioned a process in which $NH_3$ occurs as a conversion product in an enzymatic reaction such as

$$NH_2\ CO\ NH_2 + H_2O \xrightarrow{\text{Uriase}} 2NH_3 + CO_2.$$

In this case the electrolyte in the compartment 41 is $NH_4Cl$ as above and the membrane system comprises the non-homogeneous membrane, with a stratum of cuprophane facing the cell and with a dense stratum of silicone rubber, and another cuprophane membrane with the enzyme urlase between the two membranes.

WHAT WE CLAIM IS:—

1. A cell for electro-chemical analysis comprising a receptacle, a sensing electrode in said receptacle, a reference electrode in a space in said receptacle which is separated from said sensing electrode and adapted to hold an electrolyte solution, and a composite membrane electrically contacting said sensing electrode, a path for an electric current extending between the reference electrode and the sensing electrode, said composite membrane comprising at least two strata one of which has a lower density than the other and is closer to the sensing electrode than the other and the other of which has a thickness which is only a small fraction of the total thickness of the membrane.

2. A cell as claimed in claim 1, wherein said denser stratum permits the passage of molecules the size of hydrogen peroxide and substantially blocks the passage of larger molecules and ions.

3. A cell as claimed in claim 2, wherein the said denser stratum has a thickness of 0.01 to 2 $\mu$m.

4. A cell as claimed in claim 1, wherein said denser stratum permits the passage of gases and blocks the passage of ions and substantially blocks the passage of non-gaseous molecules.

5. A cell as claimed in claim 4, wherein said denser stratum has a thickness of 0.1 to 5 $\mu$m.

6. A cell as claimed in claim 2, wherein said non-homogeneous membrane comprises three strata the outer two of which exhibit lower permeability to larger molecules and ions.

7. A cell as claimed in claim 1, wherein said denser stratum faces a layer containing enzyme.

8. A cell as claimed in claim 1, wherein said denser stratum contacts an aqueous layer of enzyme contained between said stratum and a second, hydrophilic membrane kept at a distance from said stratum by at least one spacer.

9. A cell as claimed in claim 8, wherein said second membrane consists of polymerised protein, cuprophane or cellulose acetate.

10. A cell as claimed in claim 1, wherein enzyme is bonded to said stratum of higher density.

11. A cell as claimed in claim 1, wherein said denser stratum faces a hydrophilic layer in which, at least on one side, is incorporated an enzyme.

12. A cell as claimed in claim 1, wherein said denser stratum contacts a spacer containing enzyme which keeps a second membrane at a distance from said stratum, said second membrane being permeable to molecules and ions of lower molecular weight only.

13. A cell as claimed in claim 1, wherein said stratum of lower density is a hydrophilic material and said stratum of higher density is a hydrophobic material.

14. A cell as claimed in claim 1, wherein all strata in said composite membrane consist of the same hydrophilic material and at least one of said strata has a higher density obtained by a heat treatment.

15. A cell as claimed in claim 1, wherein all strata in said composite membrane consists of cellulose acetate.

16. A cell as claimed in claim 1, wherein said denser stratum consists of a silicone rubber and said stratum of lower density consists of cuprophane.

17. A cell as claimed in claim 1, wherein said composite membrane contacts the sensing electrode as well as said space holding an electrolyte solution and said reference electrode.

18. A cell as claimed in claim 1, wherein said composite membrane covers the active surface of a sensing electrode in the form of an ion sensitive electrode as well as said

space holding an electrolyte solution and said reference electrode.

19. A cell for electrochemical analysis, substantially as described with reference to the accompanying drawings.

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1 442 303
2 SHEETS

COMPLETE SPECIFICATION

This drawing is a reproduction of the Original on a reduced scale.

SHEET 1

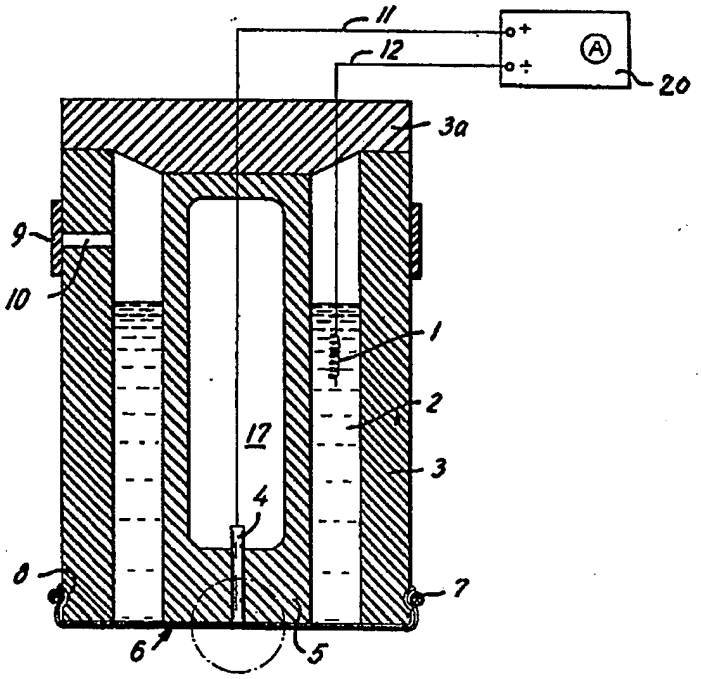


FIG. 1

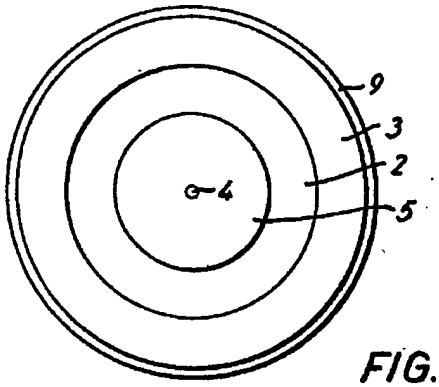


FIG. 2

1 442 303
2 SHEETS
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SHEET 2

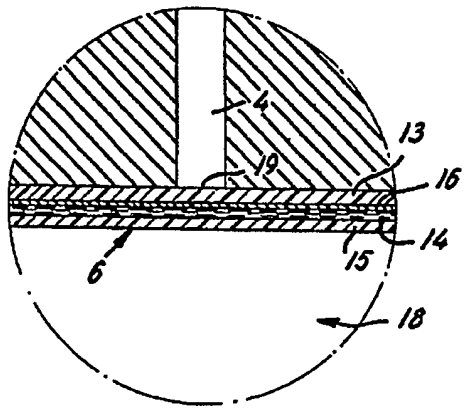


FIG. 3

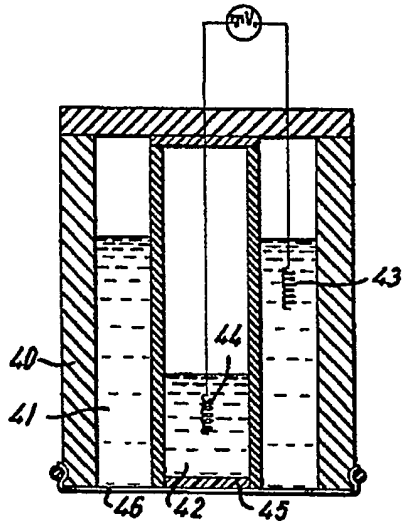


FIG. 4

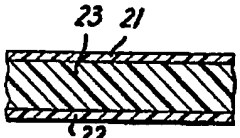


FIG. 5

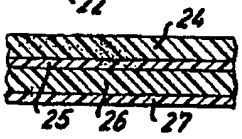


FIG. 6

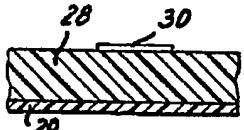


FIG. 7

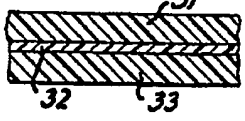


FIG. 8